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Microsatellite Variation Among Red Snapper (*Lutjanus campechanus*) from the Gulf of Mexico

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Abstract: Allelic variation at a total of 20 nuclear-encoded microsatellites was examined among adult red snapper (*Lutjanus campechanus*) sampled from 4 offshore localities in the Gulf of Mexico. The number of alleles at the 20 microsatellites ranged from 5 to 20; average (± SE) direct count heterozygosity values ranged from 0.148 ± 0.025 to 0.902 ± 0.008. No significant departures from expectations of Hardy-Weinberg equilibrium were found for any locus within samples, and genotypes at pairs of microsatellites appeared to be randomly associated, i.e., in genotypic equilibrium. Tests of homogeneity in allele distributions among the 4 localities were nonsignificant for 19 of the microsatellites. Allele distribution at microsatellite *Lca* 43 was heterogeneous among localities before (but not after) Bonferroni corrections for multiple tests executed simultaneously. Tests of homogeneity in the distribution of individual alleles at *Lca* 43 gave similar results: one low frequency allele was distributed heterogeneously among samples before, but not after, Bonferroni correction. Molecular analysis of variance indicated that more than 99% of variation at each microsatellite was distributed within sample localities. These results generally are consistent with the hypothesis of a single population (stock) of red snapper in the northern Gulf of Mexico.

Key words: Gulf red snapper, *Lutjanus campechanus,* microsatellites, population structure.

INTRODUCTION

Gulf red snapper (*Lutjanus campechanus*) in the Gulf of Mexico (hereafter Gulf) have been managed intensively since 1990, when the Gulf of Mexico Fishery Management Council (GMFMC) Reef Fish Fishery Management Plan became operative. Gulf red snapper has been an important fishery in the southeastern United States for a number of years and currently is considered overfished owing to exploitation by directed (commercial and recreational) fisheries and by high bycatch mortality of juvenile fish in the shrimp fishery (Christman, 1997). Among other issues facing management planning, including stock assessment and allocation decisions, is whether Gulf red snapper comprise a single population (stock) across the northern Gulf (MRAG Americas Inc., 1997). Management planning for Gulf red snapper within the Gulf of Mexico Exclusive Economic Zone (EEZ) and adjoining Territorial Sea currently is based on a unit (single) stock hypothesis (GMFMC, 1989, 1991). However, few data addressing the stock structure of red snapper in the Gulf were available when the original management plan was drafted. Subsequent genetic studies (A.G. Johnson, 1987, unpublished results; Gold et al., 1997; Heist and Gold, 2000) have been consistent with the existence of a single stock of Gulf red snapper, but a study by

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Bortone and Chapman (1995) suggested that genetic heterogeneity in Gulf red snapper could arise over fairly small spatial, and perhaps temporal, scales.

In this communication we report development of 15 nuclear-encoded, hypervariable genetic markers (microsatellites) generated from a genomic library of Gulf red snapper DNA. Briefly, microsatellites are rapidly evolving, short stretches of DNA composed of di-, tri-, and tetranucleotide arrays inherited in a codominant fashion (Wright and Bentzen, 1994) that have proved to be useful genetic markers of population structure in numerous taxa, including fishes (Jarne and Lagoda, 1996; Ruzzante et al., 1996; Estoup and Angers, 1998). Because new alleles at microsatellite loci appear to arise rapidly (Schug et al., 1998), the spatial distribution of alleles in a population may reflect short-term gene flow (O'Connell and Slatkin, 1993), meaning that microsatellites may reveal population structure at small spatial and temporal scales (Ruzzante et al., 1996). Allele distributions at the 15 microsatellites were documented among samples of adult red snapper from 4 offshore localities spanning the northern Gulf. Data were combined with those in a previous study (Heist and Gold, 2000) that documented allele distributions at 5 microsatellites among these same samples. Tests of allele frequency homogeneity, including molecular analysis of variance, were employed to examine genetic diversity and to test the (null) hypothesis of genetic homogeneity among the 4 samples.

Materials and Methods

To generate the microsatellites, genomic DNA libraries of red snapper DNA fragments (400–1000 bp in size) were constructed via standard procedures in our laboratory (Broughton and Gold, 1997; Turner et al., 1998; Heist and Gold, 2000). These included restriction enzyme digestion and fragment separation; ligation of fragments into a multicloning site in pBluescript II KS^+; bacterial cell transformation; and insert identification via blue/white colony selection on LB agar plates with ampicillin, IPTG, and X-gal. Library screening employed a Beckman Biomek 2000 workstation. Individual colonies were spotted twice to eliminate false positives. We also employed approaches for producing "enriched" microsatellite libraries (Walbieser, 1994). These approaches involved binding genomic DNA fragments to biotinylated oligonucleotide probes that contained desired repeat motifs. Biotinylated probes with their attached genomic fragments were bound to streptavidin-coated paramagnetic particles that held the complexes in place; nonbound genomic fragments that lacked repeat motifs were washed away. The bound fragments were then chemically released and cloned into "enriched" libraries. These enriched libraries contained a high percentage of microsatellites, thereby increasing the efficiency of development and screening.

Radiolabeled di-, tri-, and tetranucleotide probes (including $[CA]_{15}$, $[GA]_{10}$, $[ATT]_7$, $[CCT]_7$, $[GCAC]_5$, $[GAGC]_5$, and $[GACA]_8$) were used to identify candidate microsatellites. Following size estimation of DNA inserts, 152 candidate microsatellites were sequenced using an Applied Biosystems (Perkin-Elmer, Foster City, Calif.) 377 automated DNA sequencer from either end or both ends by using standard M13 sequencing primers. Identification of primers from regions flanking microsatellites employed the OLIGO software package. Primers were designed according to preset criteria that would optimize both the ease of polymerase chain reaction (PCR) amplification and the potential for multiplexing. Criteria included product length, internal stability, proportion of GC content, and primer *Tm* difference.

PCR amplifications were performed under a variety of experimental conditions to optimize procedures that produced high yields of target sequence and minimized additional fragments ("stutter" bands). Experimental tractability (reproducibility, consistency, range in allele size, frequency of "stutter" bands, if present, and microsatellite polymorphism) of PCR-amplified microsatellites was evaluated by screening a panel of red snapper previously sampled from various localities in the Gulf. A total of 24 microsatellites were evaluated in this way. PCR primer sequences, the length (in base pairs) of the cloned allele, and the annealing temperature in PCR amplification for these 24 microsatellites and for 5 generated previously by Heist and Gold (2000) are given in Table 1. Nine of the microsatellites generated in this project were considered for one reason or another to be unsuitable for further use in genetic assays, leaving a suite of 20 microsatellites (including the 5 generated previously).

For assay of individual fish, genomic DNA was isolated from frozen tissues as described in Gold and Richardson (1991). Adult samples used were from among those reported in prior studies of red snapper mitochondrial DNA (Gold et al., 1997). Localities sampled (number of individuals assayed) were Panama City, Fla. (48), Dauphin Island,

TABLE 1. Nuclear Microsatellites Developed from Red Snapper (*Lutjanus campechanus)*

Microsatellite	Primer Sequence (5′→3′) (forward and reverse, respectively)	Length (bp)	Annealing temperature (^{0}C)
1. *Lca* 20*	CAA CCC TCT GGC TAG TGT CA	215	58
	ATC CTG AAG CCC TGG TTT AC		
2. *Lca* 22*	TCC ACA GGC TTT CAC TCT TTC AG	245	58
	TGC TCT TTT CTT TCC GTC ATT CC		
3. *Lca* 27	TGA GTG GCT GTG TTT TGC TG	178	58
	GTG CGT TGT GTT TGT TGG TC		
4. *Lca* 43*	ACT GAA ATG CTG CTC TCC TT	184	56
	CAC TGT TTA CTT CTT CTG TT		
5. *Lca* 59	AGA CAG CCT GAT AGA CTG	184	54
	CAA CTG CTT CTT ACT TCT ACT		
6. *Lca* 64*	CTC CAA TCC TCC TCT CAC CT	164	54
	AGT GCC CCT GAT ACA CTT GC		
7. *Lca* 91*	GCA TCC ACC CTA AAC ATT TT	138	56
	GTT CAT CAG AGC AGC ATC CT		
8. *Lca* 107	CAG TGG AAG ATG TGA GGA GTT A	111	54
	CTG CAC CAA CAG AAA CAA AGA A		
9. *Prs* 55	AGT TAG GGT TAG TCA GAG GAG	198	56
	TAA TGT CGT CAA AAA TAG TGG		
10. *Prs* 137	GCG TCT AAA CAC ACA GGA A	162	54
	TGT AGC TGT CAA TCA TCC A		
11. *Prs* 221	AGT TTG CTA ATG TCT GAG TCA CC	227	54
	CCA TTG TCT TCG CTT ACT T		
12. *Prs* 226	GCC TGC TGT CAC CTC TCC	243	58
	TGT TCC AGC CCT TGA TTA GT		
13. *Prs* 229	CAC ATT GAA CCG TTT AAC CC	129	56
	GAA ATG ATG ACC CAG CAC AG		
14. *Prs* 235	AGG GTG ACG ATG GGT GTG	241	54
	AAG TCT CTC AAA ACC CCG AA		
15. *Prs* 240	CAA GAG GGT GAT GAA TGA	202	54
	AAT GAA ATA CCC ACT GCT		
16. *Prs* 248	CCA TCA GCT CGA CCA GAC A	224	56
	AAA GAG ACA CGG CAC GGA C		
17. *Prs* 257	AAA GTT CTT GTG ATG TGT	135	54
	GAG AAA ATG TTG GAA TGA		
18. *Prs* 260	GGT AAA ATG CTC CCT TCC T	111	56
	GTG GTA GTG GGT GAA ATT CT		
19. *Prs* 275	CAC AGA TAC AAA CCC AGA CA	145	54
	AGT AGG TCT TTG GTC ATC A		
20. *Prs* 281	AAT CAG ACC AAA TGA GAT A	181	48
	GTC CAA TCT GTA ACA AAC T		
21. *Prs* 282	CAG AGG AGG CAG AAC AGA	123	54
	ACC ACA CTA ATG CAC ACA C		
22. *Prs* 291	TAA ACC CAA GGA AAC GCT CAT	126	54
	GCC GAG GGG TGA GTG AGG A		
23. *Prs* 303	ACT CTG GAG GAA TGG GTG GAA A	132	58
	TGA AGG GCT GAC AGG TGG A		

TABLE 1. Continued

Microsatellite	Primer Sequence (5′→3′) (forward and reverse, respectively)	Length (bp)	Annealing temperature (^{0}C)
24. *Prs* 304	ATG TCA TCC TGT GCT GTC	130	56
	CTA CCT GTC TGC ACT GTT		
25. *Prs* 305	CTG CAA TTA AGC CAA CTG TCA A	169	56
	TGA GAG GAC GCA ACA ATA CAA C		
26. *Prs* 328	AGG TCA TTG TGG TGG GTG TAT	202	54
	TTA CCG TCA CTT CCA GAA CAG		
27. *Prs* 333	CTA TTA GCA GGG CTC TGT GTG	149	58
	GAC TCC GAC TGA CAT TTT CAA		
28. *Prs* 352	CAG GGA ACG ACT GCT GCT AG	195	58
	GGA CGT GGG GTG TGA AGA TT		
29. *Prs* 357	TAC AGT GCC TTA TGC AAT AC	141	56
	CAT TCG TGA GAT GCA TGT		

*Microsatellites were generated previously (Heist and Gold, 2000)

Ala. (53), Galveston, Tex. (47), and Merida, Mexico (44). Genotypes at the 20 microsatellites were determined by PCR amplification and gel electrophoresis. Prior to amplification, one of the primers was kinase-labeled with γ^{32}P-ATP by T4 polynucleotide kinase (30 minutes, 37°C). PCR reactions contained approximately 5 ng of genomic DNA, 0.1 unit of *Taq* DNA polymerase, 0.5 μM of each primer, 800 μM dNTPs, 1–2 mM $MgCl_2$, 1X *Taq* buffer at pH 9.0 (Promega, Inc., Madison, Wis.), and sterile deionized water in a total volume of 10 μl. Thermal cycling was carried out in 96-well plates as follows: denaturation (45 seconds, 95°C), annealing (30 seconds, temperature as per Table 1), and polymerization (30 seconds, 72°C), for 30 cycles. Upon completion of thermal cycling, 5 μl of "stop" solution (Promega, Inc.) was added to each sample. Aliquots (3 μl) of each PCR reaction were then electrophoresed in 6% denaturing polyacrylamide ("sequencing") gels. Gels were dried and exposed to x-ray film. Alleles at individual microsatellites were scored as the size in base pairs of the fragment amplified by PCR. Genotypes at each microsatellite for each individual were scored and entered into a database.

Statistical analysis involved generation of allele frequencies and (direct-count) heterozygosity values, as well as significance testing of genotypic proportions relative to those expected under conditions of Hardy-Weinberg equilibrium. We followed recommendations in Ruzzante et al. (1996) and employed permutation tests (Manly, 1991) to estimate probability values for tests of Hardy-Weinberg equilibrium at each microsatellite within each sample. Significance levels for simultaneous tests were adjusted by using the sequential Bonferroni approach (Rice, 1989). Tests of genotypic equilibrium at pairs of microsatellites were carried out as a surrogate to assess whether any microsatellites were genetically linked. Probability values for tests of genotypic equilibrium were generated by 1000 iterations. Allele frequencies and heterozygosity values were obtained using BIOSYS-1.7 (Swofford and Selander, 1981). Tests of Hardy-Weinberg and genotypic equilibria employed the package GENEPOP (Raymond and Rousset, 1995).

Tests of genetic homogeneity among samples followed the approach used by Ruzzante et al. (1998). The underlying null hypothesis in each test (comparison) was that allele distributions are homogeneous among localities. Tests (analyses) included the Monte Carlo procedure of Roff and Bentzen (1989), as implemented in the restriction enzyme analysis package of McElroy et al. (1992), Fisher's exact tests, as implemented in GENEPOP (10,000 dememorizations, 50 batches, 1000 iterations per batch), and the molecular analysis of variance (AMOVA) of Excoffier et al. (1992). Significance of tests of genetic homogeneity also employed permutation tests (bootstrapping) with 1000 resamplings per individual comparison. Significance levels for simultaneous tests were adjusted by using the sequential Bonferroni approach (Rice, 1989). Tests of genetic homogeneity were carried out separately for each of the 20 microsatellites.

Results and Discussion

Descriptive statistics for 20 microsatellites, including allele frequencies, sample sizes, direct-count heterozygosity values, and results of tests of conformance of observed genotypes to expectations based on the Hardy-Weinberg equilibrium, for each sample locality, are given in Appendix Tables A1 and A2. Values for 4 of the microsatellites (*Lca* 20, *Lca* 22, *Lca* 64, and *Lca* 91) were reported in Heist and Gold (2000), as were values for *Lca* 43. During efforts to multiplex the microsatellites, we discovered that what initially had been dismissed as an artifact during scoring of alleles at *Lca* 43 was an allele (*Lca* 43-162) that was substantially smaller than the next-largest allele (*Lca* 43-174). Briefly, the sequence of allele 184 at *Lca* 43 is 5′-G$(TG)_8$GG-GAC$(GT)_5$ATGGTGTTTAAGTGTAGAC GTGAG$(TG)_3$TT$(TG)_4$-3′; *Lca* 43-162 has the sequence 5′-$(GT)_5$ATGGTGTTTAAGTGTAGAC GTGAG$(TG)_3$TT$(TG)_4$-3′) and is lacking 22 bp relative to the 5′ end of *Lca* 43-184. Consequently, all individuals were rescored for microsatellite *Lca* 43, accounting for the minor differences in allele frequencies between this study and Heist and Gold (2000).

The 9 microsatellites (repeat sequence of the cloned allele) not used in the survey were *Lca* 59, $[GATA]_{11}$; *Prs* 226, $[CA]_{16}$; *Prs* 235, $[AC]_{11}$; *Prs* 281, $[GT]_{21}$; *Prs* 291, $[AT]_{12}$; *Prs* 304, $[TG]_6$ TC $[TG]_7$; *Prs* 305, $[CA]_{16}$; *Prs* 352, $[CA]_{10}$; and *Prs* 357, $[GT]_{20}$. These microsatellites were omitted from the survey because of significant deviations from Hardy-Weinberg expectations in an initial survey, inconsistent amplification with the PCR primers employed, or too many alleles (or extreme size difference among alleles) for reliable scoring of phenotypes (genotypes).

Summary statistics for the 20 microsatellites are given in Table 2 and include the repeat sequence of the cloned allele, number of alleles detected, the average (direct-count) heterozygosity (± SE) observed among sample localities, and results of tests of conformance of observed genotype proportions to expectations of Hardy-Weinberg equilibrium. Virtually all of the microsatellites generated were CA (or complementary TG) dinucleotide repeats, with number of alleles per microsatellite ranging from 5 (*Lca* 20, *Prs* 260, and *Prs* 328) to 20 (*Prs* 240 and *Prs* 248). Direct-count heterozygosity, averaged over the 4 sample localities, ranged from 0.148 ± 0.025 (*Lca* 20) to 0.902 ± 0.008 (*Prs* 257). These results indicate that the 20 microsatellites generated from red snapper are typical of those found in other vertebrate organisms, including fishes (e.g., Turner et al., 1998). Following Bonferroni correction (Rice, 1989), genotype proportions at all 20 microsatellites in all 4 sample localities did not deviate significantly from proportions expected under Hardy-Weinberg equilibrium. This indicates that all 20 microsatellites should be suitable for a variety of studies on Gulf red snapper, from population structure to paternity and kinship.

Tests of genotypic equilibrium between pairs of loci were carried out both within samples and when samples were pooled across localities. Significant genotypic disequilibrium (following Bonferroni correction) within samples was found in 11 pairwise comparisons. Localities (microsatellite comparison) were Panama City, Fla. (none), Dauphin Island, Ala. (*Lca* 27–*Prs* 248, *Lca* 43–*Lca* 107, *Lca* 107–

Table 2. Summary of Microsatellite Variation in Red Snapper (*Lutjanus campechanus*) Sampled from 4 Localities in the Gulf of Mexico

Microsatellite	Repeat sequence	No. of alleles	Average heterozygosity ±SE	P_{HW}*
Lca 20†	$[CA]_9$	5	0.148 ± 0.025	0/4
Lca 22†	$[CA]_{18}$	14	0.765 ± 0.041	0/4
Lca 27	$[TG]_{19}$	19	0.761 ± 0.022	0/4
Lca 43†	Complex msat 1‡	8	0.498 ± 0.029	0/4
Lca 64†	$[CA]_{12}$	10	0.721 ± 0.037	0/4
Lca 91†	$[CA]_{12}$	8	0.502 ± 0.034	0/4
Lca 107	$[CA]_{16}$	12	0.759 ± 0.053	0/4
Prs 55	$[TG]_{13}$	8	0.248 ± 0.052	0/4
Prs 137	$[TG]_{13}$	13	0.681 ± 0.055	0/4
Prs 221	$[CA]_{10}$ CG $[CA]_3$	16	0.815 ± 0.029	0/4
Prs 229	$[CA]_8$	8	0.585 ± 0.037	0/4
Prs 240	$[CA]_{21}$	20	0.827 ± 0.038	0/4
Prs 248	$[CT]_6$ $[C]_5$ T $[CA]_{12}$	20	0.844 ± 0.008	0/4
Prs 257	$[AAG]_{15}$	17	0.902 ± 0.008	0/4
Prs 260	Complex msat 2§	5	0.371 ± 0.027	0/4
Prs 275	$[CA]_{10}$	7	0.578 ± 0.040	0/4
Prs 282	$[TG]_2$ CA $[TG]_9$	12	0.608 ± 0.041	0/4
Prs 303	$[CA]_{11}$	11	0.458 ± 0.025	0/4
Prs 328	$[TG]_9$	5	0.529 ± 0.039	0/4
Prs 333	$[GT]_4$ TG $[GT]_8$	6	0.306 ± 0.028	0/4

*Proportion of samples where $P < .05$, following Bonferroni correction.
†Microsatellites were developed by Heist and Gold (2000).
‡Complex msat 1 = G$[TG]_8$GGGAC$[GT]_5$ATGGTGTTTAAGTGTAGACGGTGAG $[TG]_3$ TT $[TG]_4$.
§Complex msat 2 = $[TG]_4$ AGTGCA $[TG]_2$ TA $[TG]_6$.

Table 3. Probability Values of Tests of Genotypic Disequilibrium Within Sample Localities for Those Pairs of Microsatellites Where Significant Genotypic Disequilibrium (following Bonferroni corrections) Was Detected When Samples Were Pooled Across Localities

Microsatellite pair	Panama City, Fla.	Dauphin Island, Ala.	Galveston, Tex.	Merida, Mexico
Lca 22–*Lca* 27	0.171	0.196	0.926	0.950
Lca 22–*Prs* 55	0.541	0.654	0.036	0.759
Lca 22–*Lca* 107	0.784	0.408	0.589	0.313
Lca 22–*Prs* 282	0.436	0.093	0.315	0.036
Lca 27–*Prs* 275	0.819	0.716	0.621	0.534
Lca 43–*Lca* 107	0.356	0.000*	0.365	0.269
Lca 43–*Prs* 137	0.002	0.062	0.185	0.366
Lca 64–*Prs* 240	0.588	0.632	0.765	1.000
Lca 91–*Prs* 221	0.195	0.779	0.124	0.742
Prs 229–*Prs* 257	0.044	0.979	0.976	0.730

*Significant probability value following Bonferroni correction.

Prs 240, *Prs* 257–*Prs* 303, *Prs* 282–*Prs* 333), Galveston, Tex. (*Lca* 64–*Lca* 107, *Prs* 248–*Prs* 303), and Merida, Mexico (*Lca* 27–*Lca* 64, *Lca* 43–*Prs* 257, *Lca* 64–*Prs* 333, *Prs* 229–*Prs* 328). Note that none of the significant comparisons was found at more than one locality. Tests carried out when samples were pooled across localities yielded 10 significant probability values following Bonferroni correction. In only one of these pairwise comparisons (between *Lca* 43 and *Lca* 107) was there a significant probability of genotypic disequilibrium within any single locality (Table 3). Collectively, these results indicate that genotypes at pairs of microsatellites appear randomly associated and suggest that all 20 microsatellites are inherited independently.

Tests of homogeneity in allele distributions among the 4 samples were nonsignificant before and after Bonferroni correction at 19 of the microsatellites (Table 4). Significant probability values at *Lca* 43 were obtained before Bonferroni correction with the Roff-Bentzen Monte Carlo method ($P = .016$) and Fisher's exact test ($P = .005$), but not with AMOVA ($\phi_{ST} = .008$, $P = .078$). Neither significant result remained after Bonferroni correction. To examine this further we carried out "V" tests (DeSalle et al., 1987) on arcsine square-root transformed frequencies of each allele at *Lca* 43. Only 1 allele (*Lca* 43-162) was distributed heterogeneously ($P \approx .027$) among the 4 sample localities. Again, the result was significant before, but not after, Bonferroni correction. *Lca* 43-162 is not the most common allele at *Lca* 43 (frequencies range from 11.0% in the sample from Merida, Mexico, to 1.1% in the sample from Galveston, Tex.), and the distribution of *Lca* 43-162 among the sample localities does not follow any sort of spatially linear trend (Appendix Table A1). This, along with the expectation that 1 in 20 (of the original) tests may be significant (at $\alpha = .05$) by chance alone, leads us to conclude that there is no biologically meaningful heterogeneity at *Lca* 43. We also employed the Fisher (1954) method of combining probabilities over (multiple) independent tests of significance for each of the three methods. Combined probability values were .092 (Roff-Bentzen procedure), .073 (exact tests), and .793 (from AMOVA). This supports further the hypothesis of genetic homogeneity among the 4 sampled localities and, interestingly, suggests that the AMOVA may be less powerful than the other two tests of genetic homogeneity. Finally, ϕ_{ST} values (derived from AMOVA), an index of the proportion of the genetic variation distributed among localities, ranged from 0 (including negative values) to 0.009, meaning that the overwhelming majority (>99%) of the variation at these microsatellites was distributed within localities.

Given that the samples of Gulf red snapper were from localities that span the northern Gulf (Panama City, Fla., to Galveston, Tex.) and include a sample from the northern Yucatan Peninsula, these results are consistent with the hypothesis of a single population (stock) of Gulf red snapper in the northern (and western) Gulf of Mexico. There are, however, caveats to this hypothesis. The first, generally acknowledged by most authors (e.g., Camper et al., 1993; Gold and Richardson, 1998), is that one cannot prove a null hypothesis; a finding that geographic samples do not differ in allele frequencies could mean simply that each sample has the same parametric allele frequency at each genetic

Table 4. Results of Tests for Spatial Homogeneity in Allele Distributions at 20 Microsatellites Among 4 Samples of Red Snapper (*Lutjanus campechanus*) from the Gulf of Mexico

Microsatellite	P_{RB}*	P_{EXACT}†	Φ_{ST}‡	*P*
Lca 20	.317	.457	−.001	.470
Lca 22	.622	.538	−.003	.793
Lca 27	.159	.421	.002	.303
Lca 43	.016	.005	.008	.078
Lca 64	.295	.237	.001	.328
Lca 91	.306	.119	.001	.317
Lca 10	.275	.164	.002	.285
Prs 55	.127	.121	.009	.073
Prs 137	.321	.506	−.004	.783
Prs 221	.205	.221	−.004	.843
Prs 229	.796	.893	−.004	.773
Prs 240	.154	.283	.000	.425
Prs 248	.369	.402	.000	.424
Prs 257	.746	.736	−.001	.662
Prs 260	.768	.734	−.008	.865
Prs 275	.729	.654	−.005	.754
Prs 282	.244	.387	−.002	.618
Prs 303	.579	.545	−.007	.920
Prs 328	.037	.038	.001	.319
Prs 333	.398	.525	−.007	.902

*P_{RB}: based on 1000 bootstrapped replicates (after Roff and Bentzen, 1987).

†P_{EXACT}: based on Fisher's exact tests, with 1000 permutations.

‡Φ_{ST}: estimate of population subdivision based on molecular analysis of variance (AMOVA) after Excoffier et al. (1992); *P* represents the probability that Φ_{ST} differs significantly from 0 (5000 permutations).

marker. A second caveat is the possibility that the observed genetic homogeneity reflects past (historical), rather than present-day, population structure. As discussed by Gold and Richardson (1998), subpopulations (stocks) could be currently isolated, at least partially, yet have undergone sufficient gene flow in the recent past such that they remain indistinguishable in allele frequencies.

Both of the above caveats will accompany virtually any assessment of population structure that uses genetic markers. However, the number of independent genetic markers used and the rate at which new alleles appear at these markers affect the constraints imposed by each of the two caveats. Because each independent genetic marker represents a separate test of the null hypothesis, increasing the number of such markers is expected to increase the overall power to reject a false null. In this case we employed 20, putatively independent microsatellites and found only 1 microsatellite (*Lca* 43) at which 1 low-frequency allele might be distributed heterogeneously among the 4 geographic samples of Gulf red snapper. As to the caveat of confounding events in the recent past that might overshadow present-day population structure, microsatellites are considered to be among the best, co-dominantly inherited genetic markers to asses contemporaneous population structure because of their high rates of new allele formation relative to other genetic markers (O'Connell and Slatkin, 1993; Ruzzante et al., 1996). Thus, while we cannot falsify the potentially confounding effect of historical events, the use of 20 independently inherited microsatellites indicates that power to detect contemporaneous population structure is near optimal.

A third caveat to the inference, based on these microsatellite data, that Gulf red snapper comprise only a single population (stock) in the northern and western Gulf is that our samples undoubtedly included individuals from different cohorts (year classes). We attempted at the time to obtain similarly sized individuals (range, 35–45 cm in fork length) under the assumption that individuals primarily would be from one or two cohorts. Recent work by C.A. Wilson and D.L. Nieland (manuscript submitted), however, has demonstrated that age-length relationships in Gulf red snapper are not necessarily straightforward. Individuals in the size range of 35 to 45 cm in fork length primarily would be comprised of age 2 and age 3 fish but also could include individuals as old as age 8. Our sampling also was not restricted to individuals in the size range of 35 to 45 cm in fork length. The issue here is that movement of adults from one putative subpopulation (stock) to another could confound efforts to identify individuals of either subpopulation (stock), particularly if there is any tendency to natal philopatry. We currently are undertaking a study of age 0 Gulf red snapper to mitigate this problem. However, on the basis of the majority of genetic data at hand, including both mitochondrial DNA restriction sites (Camper et al., 1993; Gold et al., 1997) and microsatellites (Heist and Gold, 2000; this study), the best working hypothesis for management of the Gulf red snapper resource is that there is a single population (stock) of Gulf red snapper in the Gulf of Mexico.

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Appendix Table A1. Allele Frequencies at 20 Microsatellites in Red Snapper (*Lutjanus campechanus*) Sampled from 4 Localities in the Gulf of Mexico

	Sample locality (adults)			
Microsatellite (allele*)	Panama City, Fla.	Dauphin Island, Ala.	Galveston, Tex.	Merida, Mexico
Lca 20†				
207	.000	.000	.000	.011
211	.000	.000	.021	.000
213	.042	.047	.064	.045
215	.938	.953	.883	.920
217	.021	.000	.032	.023
Lca 22†				
231	.020	.000	.011	.000
233	.010	.000	.011	.000
235	.449	.481	.415	.409
236	.031	.019	.021	.034
237	.000	.028	.043	.034
239	.194	.217	.266	.205
241	.143	.075	.074	.114
243	.041	.057	.085	.068
245	.031	.038	.043	.045
247	.041	.019	.000	.057
249	.020	.028	.000	.023
251	.010	.038	.032	.000
252	.000	.000	.000	.011
255	.010	.000	.000	.000
Lca 27				
160	.000	.010	.000	.000
162	.041	.038	.044	.036
164	.014	.000	.000	.012
166	.000	.000	.000	.012
168	.000	.010	.000	.024
170	.095	.087	.089	.143
172	.203	.125	.100	.190
174	.297	.423	.400	.321
176	.054	.048	.122	.060
178	.108	.135	.100	.071
180	.081	.048	.044	.095
182	.014	.029	.022	.012
184	.041	.000	.000	.000
186	.000	.019	.033	.012
188	.014	.019	.011	.000
190	.027	.000	.000	.000
192	.000	.000	.011	.000
194	.014	.000	.000	.000
196	.000	.010	.022	.012
Lca 43				
162	.076	.043	.011	.110
176	.011	.106	.011	.049
178	.000	.000	.000	.012
184	.174	.117	.133	.049
186	.630	.670	.756	.671
188	.087	.043	.056	.073
190	.011	.000	.000	.012
192	.011	.021	.033	.024
Lca 64†				
158	.021	.010	.011	.000
160	.000	.010	.011	.000
162	.011	.010	.021	.000
164	.287	.230	.309	.295
166	.287	.210	.213	.216
168	.245	.310	.330	.216
170	.085	.100	.074	.159
172	.032	.110	.011	.080
174	.011	.000	.021	.023
176	.021	.010	.000	.011
Lca 91†				
130	.000	.000	.000	.011
132	.000	.010	.011	.000
134	.000	.038	.011	.023
136	.413	.462	.511	.489
138	.533	.490	.415	.398
140	.033	.000	.053	.057
142	.011	.000	.000	.011

Appendix Table A1. Continued

Microsatellite (allele*)	Sample locality (adults) Panama City, Fla.	Dauphin Island, Ala.	Galveston, Tex.	Merida, Mexico
144	.011	.000	.000	.011
Lca 107				
97	.034	.010	.011	.012
99	.052	.040	.068	.081
101	.259	.230	.307	.267
103	.310	.410	.273	.360
105	.069	.040	.080	.081
107	.034	.130	.080	.070
109	.155	.040	.102	.093
111	.017	.010	.023	.035
113	.052	.080	.045	.000
115	.000	.000	.011	.000
117	.000	.010	.000	.000
119	.017	.000	.000	.000
Prs 55				
192	.000	.010	.011	.000
194	.000	.000	.000	.012
196	.014	.038	.022	.071
198	.811	.894	.911	.857
200	.135	.029	.044	.048
202	.027	.019	.011	.000
204	.014	.010	.000	.000
210	.000	.000	.000	.012
Prs 137				
158	.000	.000	.011	.012
160	.000	.019	.011	.000
162	.109	.144	.174	.134
166	.000	.000	.033	.000
168	.031	.010	.033	.024
170	.453	.481	.435	.402
172	.000	.048	.011	.024
174	.297	.260	.217	.305
176	.063	.010	.043	.073
178	.016	.019	.033	.024
180	.016	.000	.000	.000
188	.016	.000	.000	.000
190	.000	.010	.000	.000
Prs 221				
223	.029	.020	.043	.058
225	.103	.059	.096	.023
227	.176	.147	.160	.151
229	.324	.324	.309	.349
231	.000	.010	.021	.023
233	.000	.000	.021	.047

Appendix Table A1. Continued

Microsatellite (allele*)	Sample locality (adults) Panama City, Fla.	Dauphin Island, Ala.	Galveston, Tex.	Merida, Mexico
235	.250	.304	.223	.279
237	.074	.078	.064	.012
239	.000	.010	.021	.012
241	.000	.010	.000	.023
243	.000	.000	.000	.012
249	.000	.000	.021	.012
251	.029	.029	.000	.000
255	.000	.010	.000	.000
257	.000	.000	.021	.000
259	.015	.000	.000	.000
Prs 229				
121	.015	.000	.000	.000
123	.000	.010	.032	.012
127	.706	.692	.660	.605
129	.103	.154	.117	.140
131	.044	.038	.053	.081
133	.132	.087	.128	.140
135	.000	.010	.011	.023
137	.000	.010	.000	.000
Prs 240				
184	.000	.000	.000	.012
186	.014	.000	.000	.000
188	0.57	.038	.043	.070
190	.043	.038	.111	.023
192	.029	.019	.022	.012
194	.100	.067	.043	.093
196	.000	.019	.011	.058
198	.057	.067	.054	.070
200	.029	.067	.011	.012
202	.271	.288	.370	.244
204	.086	.077	.098	.070
206	.100	.154	.087	.070
208	.043	.048	.076	.058
210	.029	.010	.076	.023
212	.029	.048	.011	.058
214	.071	.038	.033	.035
216	.000	.000	.011	.023
218	.029	.019	.000	.023
220	.000	.000	.011	.047
224	.014	.000	.033	.000
Prs 248				
218	.000	.010	.000	.000
220	.014	.010	.000	.000
222	.000	.019	.000	.000

Appendix Table A1. Continued

Microsatellite (allele*)	Sample locality (adults) Panama City, Fla.	Dauphin Island, Ala.	Galveston, Tex.	Merida, Mexico
224	.114	.135	.044	.093
226	.029	.019	.000	.023
228	.229	.260	.322	.314
230	.171	.173	.100	.105
232	.057	.058	.033	.081
234	.129	.087	.122	.116
236	.129	.038	.100	.047
238	.029	.048	.078	.105
240	.057	.048	.089	.058
242	.029	.029	.044	.035
244	.000	.010	.044	.000
246	.000	.019	.000	.000
248	.000	.010	.000	.000
250	.014	.019	.011	.012
252	.000	.000	.011	.000
256	.000	.010	.000	.000
258	.000	.000	.000	.012
Prs 257				
108	.000	.000	.011	.023
111	.114	.077	.053	.093
114	.057	.067	.085	.058
117	.243	.154	.149	.163
120	.057	.067	.074	.023
123	.114	.096	.096	.116
126	.029	.096	.053	.093
129	.043	.087	.096	.151
132	.100	.106	.106	.047
135	.043	.096	.053	.070
138	.114	.077	.074	.047
141	.043	.019	.053	.081
144	.029	.029	.043	.012
147	.014	.010	.021	.012
150	.000	.019	.021	.000
153	.000	.000	.011	.000
156	.000	.000	.000	.012
Prs 260				
111	.797	.800	.818	.767
113	.000	.010	.000	.000
117	.141	.160	.125	.140
123	.063	.030	.045	.093
129	.000	.000	.011	.000
Prs 275				
123	.014	.000	.000	.000
139	.000	.010	.000	.000
141	.014	.000	.011	.012
143	.071	.048	.096	.128
145	.571	.529	.521	.512
147	.300	.385	.309	.314
149	.029	.029	.064	.035
Prs 282				
115	.000	.029	.000	.035
121	.329	.231	.255	.209
123	.557	.510	.564	.535
125	.000	.019	.021	.058
127	.057	.096	.053	.035
129	.014	.058	.064	.081
131	.000	.010	.000	.023
133	.000	.000	.011	.000
135	.000	.029	.011	.012
137	.000	.000	.011	.000
139	.029	.019	.011	.012
141	.014	.000	.000	.000
Prs 303				
128	.015	.038	.096	.070
130	.765	.750	.734	.744
132	.162	.135	.138	.128
134	.015	.019	.011	.023
136	.029	.029	.000	.000
138	.000	.019	.011	.012
140	.000	.000	.011	.000
142	.015	.000	.000	.000
144	.000	.000	.000	.012
148	.000	.010	.000	.000
152	.000	.000	.000	.012
Prs 328				
200	.029	.000	.000	.000
202	.414	.412	.351	.476
204	.543	.510	.585	.464
206	.000	.078	.064	.036
208	.014	.000	.000	.024
Prs 333				
145	.028	.010	.000	.012
149	.819	.808	.830	.837
151	.111	.144	.096	.116
153	.028	.019	.053	.000
155	.014	.019	.021	.012
157	.000	.000	.000	.023

*Allele number represents the size in base pairs of the fragment amplified.
†Reported previously in Heist and Gold (2000).

Appendix Table A2. Descriptive Statistics of Each of 20 Microsatellites Among 4 Geographic Samples of Adult Red Snapper (*Lutjanus campechanus*) from the Gulf of Mexico

Microsatellite*	Sample locality (adults)			
	Panama City, Fla.	Dauphin Island, Ala.	Galveston, Tex.	Merida, Mexico
Lca 20				
n	48	53	47	44
H_{DC}	0.125	0.094	0.213	0.159
P_{HW}	1.000	1.000	0.253	1.000
Lca 22				
n	49	53	47	44
H_{DC}	0.816	0.642	0.809	0.795
P_{HW}	0.926	0.161	0.746	0.519
Lca 27				
n	37	52	45	42
H_{DC}	0.730	0.731	0.822	0.762
P_{HW}	0.064	0.247	0.222	0.019
Lca 43				
n	46	47	45	41
H_{DC}	0.522	0.489	0.422	0.561
P_{HW}	0.403	0.483	0.025	0.504
Lca 64				
n	47	50	47	44
H_{DC}	0.638	0.800	0.766	0.682
P_{HW}	0.058	0.274	0.363	0.083
Lca 91				
n	46	52	47	44
H_{DC}	0.500	0.481	0.596	0.432
P_{HW}	0.368	0.157	0.779	0.023
Lca 107				
n	29	50	44	43
H_{DC}	0.862	0.660	0.841	0.674
P_{HW}	0.072	0.078	0.096	0.035
Prs 55				
n	37	52	45	42
H_{DC}	0.378	0.173	0.156	0.286
P_{HW}	1.000	0.134	0.278	1.000
Prs 137				
n	32	52	46	41
H_{DC}	0.844	0.596	0.652	0.634
P_{HW}	0.789	0.389	0.125	0.765
Prs 221				
n	34	51	47	43
H_{DC}	0.853	0.745	0.872	0.791
P_{HW}	0.827	0.053	0.743	0.589
Prs 229				
n	34	52	47	43
H_{DC}	0.529	0.519	0.617	0.674
P_{HW}	0.869	0.957	0.966	0.386
Prs 240				
n	35	52	46	43
H_{DC}	0.886	0.846	0.717	0.860
P_{HW}	0.547	0.104	0.312	0.088
Prs 248				
n	35	52	45	43
H_{DC}	0.829	0.865	0.844	0.837
P_{HW}	0.069	0.836	0.914	0.783
Prs 257				
n	35	52	47	43
H_{DC}	0.886	0.923	0.894	0.907
P_{HW}	0.710	0.779	0.034	0.746
Prs 260				
n	32	50	44	43
H_{DC}	0.344	0.380	0.318	0.442
P_{HW}	0.783	0.485	0.811	0.879
Prs 275				
n	35	52	47	43
H_{DC}	0.486	0.538	0.638	0.651
P_{HW}	0.456	0.662	0.037	0.948
Prs 282				
n	35	52	47	43
H_{DC}	0.486	0.635	0.660	0.651
P_{HW}	0.183	0.313	0.589	0.156
Prs 303				
n	34	52	57	43
H_{DC}	0.471	0.385	0.489	0.488
P_{HW}	0.845	0.030	0.652	1.000
Prs 328				
n	35	51	47	42
H_{DC}	0.457	0.471	0.617	0.571
P_{HW}	0.301	0.184	0.032	1.000
Prs 333				
n	36	52	47	43
H_{DC}	0.306	0.385	0.255	0.279
P_{HW}	0.285	0.824	0.135	0.692

*n = number of individuals; H_{DC} = heterozygosity (direct count); P_{HW} = probability of conformance to expected Hardy-Weinberg proportions, based on Fisher's exact test (1000 permutations).